#2488 **CD68/CD31 Immunohistochemistry Double Stain Demonstrates Increased Accuracy in Diagnosing Antibody-Mediated Rejection in Cardiac Transplant Patients.**

Carolyn Glass (Fellow, UT Southwestern) Carolyn.Glass@UTSouthwestern.edu

Background: Antibody mediated rejection (AMR) occurs in 10%-20% of cardiac transplant patients and is associated with increased mortality. The endomyocardial biopsy, used to identify microvascular injury with intravascular macrophages, activated endothelial cells, immunohistochemical (IHC) evidence of complement deposition, and/or > 10% intravascular macrophages within capillaries, remains the primary diagnostic tool for AMR. However, as recently addressed at the XIIIth Banff Allograft Pathology Conference, identifying the intravascular location of macrophages by routine histology can present diagnostic challenges. This prompted us to perform a screen of cardiac transplant cases to determine if double labeling with an endothelial and histiocytic marker could improve diagnostic accuracy.

Design: Twenty-two cardiac transplant endomyocardial biopsies previously diagnosed at a high-volume transplant center as pAMR-2 based on histologic evidence of endothelial activation, endothelial deposition with C3d or C4d or >10% intravascular macrophages, were screened using a CD68/CD31 IHC double stain. To determine whether the diagnosis of pAMR-2 would be altered using the double stain, CD68 positive intravascular macrophage percentages were calculated and retrospectively compared in the same cases diagnosed using CD68 IHC alone.

Results: The CD68/CD31 double stain showed 13 of 22 (59%) cases which had been previously been diagnosed as >10% intravascular macrophages using a CD68 IHC stain alone, had 30%, >20% and >10% in 26%, 12% and 23% of cases, respectively. The mean C4d positivity by immunofluorescence was 75% (N=12), 37% for C3d by IHC (N=8), and 30% for >10% CD68 by IHC (N=22). The patients (mean age 51 years, 27% female) had 45 months post-transplant follow up, and one third of patients had pre-transplant left ventricular assist devices.

Conclusions: Based on our institution's experience at a high cardiac transplant volume center, over a third of patients were over-diagnosed as pAMR-2 using CD68 by IHC alone. We demonstrate here the value of using a CD68/CD31 double stain to accurately determine the percent of intravascular macrophages to diagnose the "I" component of pAMR-2.